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TESTING WHEAT FOR PROTEIN WITH A RECOMMENDED METHOD FOR MAKING THE TEST

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INTRODUCTION

During the last few years the protein content has become of increasing importance in determining the price paid for any particular grade of wheat. It has not been uncommon for grain firms to pay a premium of 5 to 20 cents per bushel over the prevailing option for wheat containing a high percentage of protein. One case is known where wheat containing 18.6 per cent of protein brought 65 cents per bushel premium over ordinary wheat of the same numerical grade but which contained only 11.5 per cent of protein. When the supply of high-protein wheats is plentiful the premiums for high protein are much less than when the supply of such wheat is scarce. The Grain Market Review for March 13, 1926, issued by the United States Department of Agriculture, quoting average prices for wheat, states that "hard red spring wheat on the Minneapolis market sold at about 12 to 15 cents over the May price for 12 per cent protein No. 1 dark northern. Twelve and one-half per cent protein was quoted

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at 13 to 16 cents over the May price; 13 per cent protein was quoted at 14 to 15 cents over. At Kansas City, hard winter 12 per cent protein sold at 10 to 11 cents over the May price, 12½ per cent at 11 to 12 cents over and 13 per cent 11½ to 12½ cents over."

The practice of paying premiums for high-protein wheat is due, in a general way, to the fact that the value of flour for baking purposes is known to be related in part to the quantity of the gluten proteins present in the flour.

As the gluten proteins constitute a fairly definite proportion of the total protein content of the wheat, 75 to 80 per cent, and as there is a close relationship between the gluten content of the wheat and that found in the flour milled therefrom, the quantity of crude protein as determined by the Kjeldahl method or its modifications is a fairly dependable index of the quantity of the gluten protein present.

In addition, since there has never been a single factor test to denote baking quality, and the crude protein test is capable of being expressed in definite mathematical terms, in percentage by weight, grain dealers, millers, and bakers have come to use the protein test as an aid in the valuation of wheat and flour. At present, the hard red spring and hard red winter wheats are marketed on a protein basis.

On certain occasions when the supply of high-protein wheat has been less than the demand, premiums of as much as a cent have been paid for each added tenth of 1 per cent of protein over a given basic amount. To trade on so close a margin taxes the protein test to its uttermost and is the cause of many of the complaints as to inaccuracy of chemists in making their determinations.

To determine how close different laboratories should be able to report results of protein tests on the same sample of wheat, the methods and technique used in making protein tests were studied in mill laboratories, commercial protein-testing laboratories and grain-inspection department laboratories in the Central Northwest and Southwest. The results of this survey are incorporated in this bulletin as well as the report of an exhaustive laboratory study relating to the value of many of the outstanding methods and technique used by the different protein-testing laboratories.

A standard method for making protein tests is described. Recommendations are given for eliminating sources of error, so that concordant results between different laboratories can be obtained.

PROTEIN TESTS BY VARIOUS LABORATORIES

In an investigation to determine the reasons for non-uniformity or variations in protein results as made by different protein-testing laboratories 12 bulk samples of wheat representing the hard red spring and hard red winter crops were thoroughly cleaned, mixed, divided into approximately 50-gram portions and placed in sealed air-tight containers to insure uniformity of moisture on arrival at the various testing laboratories. Samples 1 to 6 represented wheats of the hard red winter class and samples 7 to 12 represented wheats of the hard red spring crop.

Portions of each of these 12 samples were sent to 45 laboratories with a request for a report on their protein content. Four types of

laboratories were reached: Research or experimental, mill, elevator, and commercial.

Data obtained from this cooperative study are summarized in Table 1. A study of this table throws some interesting light upon the accuracy of making protein tests by different laboratories.

TABLE 1.—*Summary of crude protein results obtained at various laboratories on 12 samples of wheat*

	Sample number											
	1	2	3	4	5	6	7	8	9	10	11	12
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Average	10.62	11.75	12.76	12.59	12.65	15.40	12.26	12.78	14.30	10.40	10.55	11.07
Maximum	11.00	12.14	13.16	13.00	13.11	16.04	12.60	13.26	14.68	10.85	11.01	11.70
Minimum	10.34	11.36	12.40	12.42	12.21	12.89	11.43	12.04	13.46	10.00	10.26	10.65
Range	0.66	0.78	0.76	0.58	0.90	[1] 3.15	1.17	1.22	1.22	0.85	0.75	1.05

[1] This figure is believed to be an error in reporting.

With one exception, the maximum difference found with any one sample was 1.22 per cent; the minimum difference was 0.58 per cent. These figures are much larger than are usually associated with good laboratory technique in making protein tests, and are considerably larger than that allowed by either the Kansas City or the Omaha boards of trade. They allow 0.12 and 0.20 per cent, respectively.

In the case of sample 6, one laboratory reported a test that varied 3.15 per cent from the maximum test found by the other chemists. It is believed that there must have been a mistake in the calculations made by this laboratory, as the other analyses made by this laboratory were within the ranges obtained by the other laboratories. This test will be considered as an error in reporting and will not be included in the discussion.

There was a greater range in results with wheats of the hard red spring class than with wheats of the hard winter class.

The size of the samples ground to make the protein test varied at the different laboratories from 6 to 60 grams. One collaborator did not grind his sample, but made the test on the whole grain. Roughly speaking, 65 per cent of the laboratories digested a 1-gram sample and 35 per cent digested a 2-gram sample. The quantity of acid used in the digestion mixture varied from 15 to 25 cubic centimeters with the laboratories using a 1-gram sample. Thirty per cent of those digesting a 1-gram sample used 25 cubic centimeters; 25 per cent, 15 to 18 cubic centimeters; and 38 per cent, 20 cubic centimeters. Of the laboratories using a 2-gram sample for digestion, 40 per cent used 25 cubic centimeters of acid; 40 per cent, 30 cubic centimeters; and 18 per cent, 20 cubic centimeters.

The catalytic agents used were numerous—sodium sulphate, potassium sulphate, metallic mercury, yellow oxide of mercury, red oxide of mercury, mercuric sulphate, metallic copper, and copper sulphate. Approximately 63 per cent of the laboratories used sodium sulphate in quantities varying from 3 to 15 grams; 35 per cent used potassium sulphate in the same quantities. Considering the mercuric catalysts, 18 per cent used metallic mercury; 10 per cent,

yellow oxide of mercury; 8 per cent, red oxide of mercury; and 18 per cent, mercuric sulphate. The lowest quantity of mercury used was 0.5 gram and the highest was 1 gram. Copper sulphate in quantities varying from 0.05 gram to 2 grams was used by 47 per cent of the laboratories. The great majority used approximately 0.2 gram. Three collaborators used metallic copper in the same ratio that copper sulphate was being used.

The Kjeldahl [1] method or modifications thereof was used by 24 per cent of the laboratories. Forty per cent used the Gunning [2] method; 18 per cent, the method developed by the Kansas City Protein Referee Board,[3] and the remainder used methods peculiar to themselves. The time of digesting the samples varied from 25 to 180 minutes. Both electricity and gas were used. The average time consumed with the gas heaters was 75 minutes; with the electric heaters, 50 minutes. Although the differences are not great, a greater range and a higher average protein content were found by the laboratories using electricity than by those using gas.

Three receiving acids were used—sulphuric, hydrochloric, and boric. Expressed as acid sufficient to hold 1 milligram of nitrogen, a sufficient quantity was used to hold from 14 to 122.5 milligrams. The strength of the hydrochloric and sulphuric acids varied from N/14 to N/2, whereas the usual 4 per cent solution of boric acid was used. When it is known that wheat will vary in protein content from 7 to approximately 20 per cent, it is evident that acid equivalent to 35 milligrams of nitrogen must be present to hold the nitrogen released from all types of samples, when a 1-gram sample is digested. These data show that few of the laboratories that submitted figures on this subject are using insufficient acid to hold these quantities of nitrogen.

As so many variables entered into the final analysis, it was almost impossible to eliminate and study each factor separately to determine which were responsible for the differences reported in the collaborative studies. Investigations were therefore planned to uncover the causes of some of the differences. These investigations were carried forward in two sections, the first having to do with the methods of digesting the samples, and the second relating to the technic of distilling. The outstanding points studied were: Analytical methods, time of digestion, quantity and nature of the catalytic agents, correct standardization of the receiving or titrating solutions, procuring and preparation of the sample for analysis, nature and volume of the receiving acids, and care in distillation. The digesting and distilling apparatus used in these studies are shown in Figures 1 and 2.

DIGESTION STUDIES

In planning this work, recognition was given to the importance of time of digestion. It is evident that this will vary, depending upon the intensity of the heat applied. For these studies, therefore, three different intensities of heat were chosen. These are described

[1] Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists. Revised to July 1, 1924, p. 6, par. 17.

[2] Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists. Revised to July 1, 1924, p. 8, par. 20.

[3] HERMAN, R. S. A PROTEIN REFEREE BOARD. Southwestern Miller, vol. 3, No. 31, p. 35, 1925.

as low, medium, and high heat. The standards for these heats were established as follows: 200 cubic centimeters of distilled water were placed in a 500-cubic-centimeter Kjeldahl flask similar to those used in the digestion process, and brought to a boil. The heaters and burners were so adjusted that 50 cubic centimeters of water evapor-

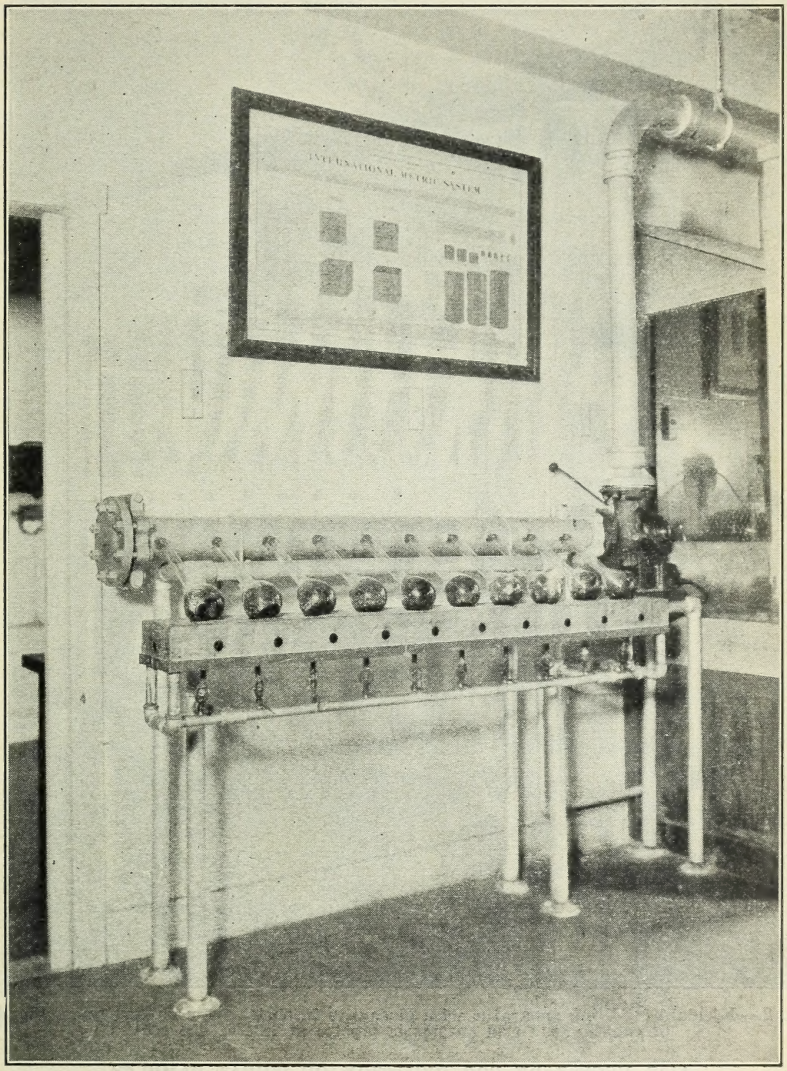

FIG. 1.—Kjeldahl digesting apparatus for the determination of protein in cereal and cereal products. Digestion flasks, with material in position

ated with the low heat in 20 minutes; 100 cubic centimeters with the medium heat; and 150 cubic centimeters with the high heat. To keep the heat intensities at the same degree, so that they would not vary from time to time and from day to day, each gas burner was set to give the same amount of heat, and this heat was kept constant by

means of a gas-pressure regulator.[4] Frequent check tests were made to keep the heat constant.

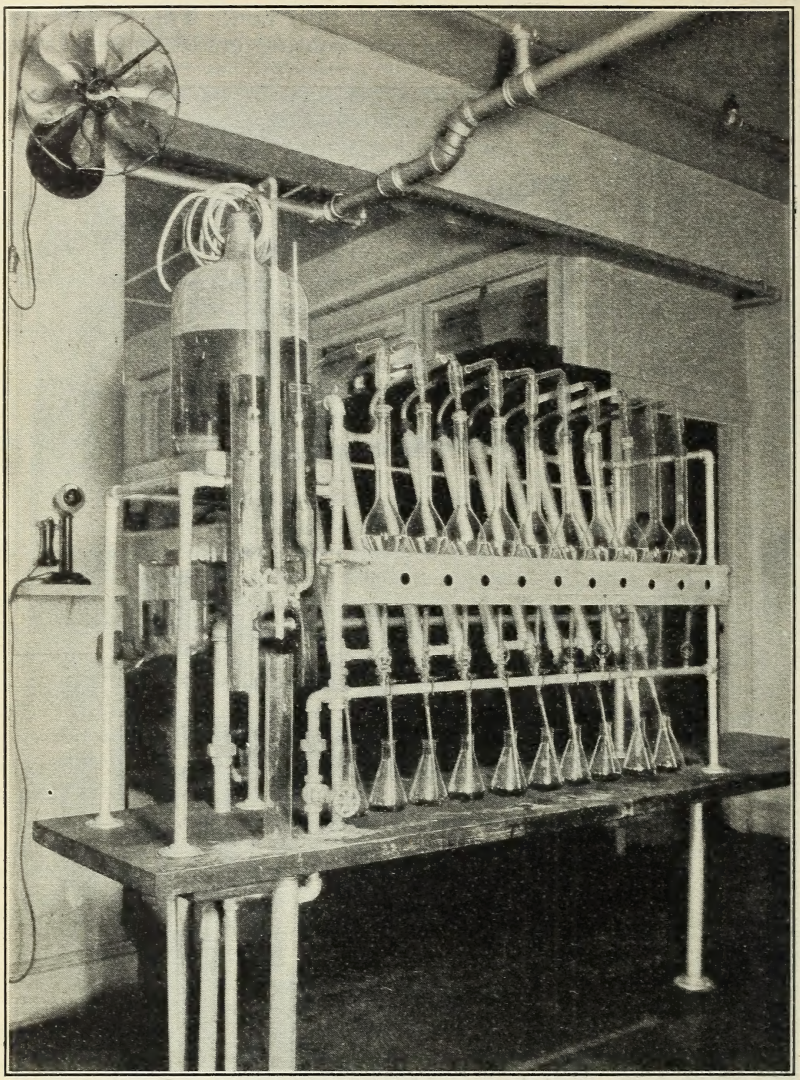

FIG. 2.—Kjeldahl distilling apparatus with glassware in position for making test. Supply of caustic soda and automatic pipette at left of the still

ACID NECESSARY FOR DIGESTION OF WHEAT SAMPLES

The acid used by the various collaborators varied considerably, regardless of size of sample. Experiments were made, therefore, to determine how much acid was actually needed to digest wheat samples completely.

[4] COLEMAN, D. A. and BOERNER, E. G. THE BROWN-DUVEL MOISTURE TESTER AND HOW TO USE IT. Dept. Bul. 1375, p. 22. 1926.

Into 500-cubic-centimeter volumetric flasks were measured 20-cubic-centimeter portions of sulphuric acid, specific gravity 1.82, and made to volume. The acid present was determined by titrating against a standard solution of potassium hydroxide. Twenty-cubic-centimeter portions of the same acid were then placed in 500-cubic-centimeter Kjeldahl flasks with 10 grams of K_2SO_4 and 0.7 gram yellow mercuric oxide, and the mixture was heated for the time necessary to digest a sample of wheat. This time varied with the heat intensity: With low heat, two hours; with medium heat, an hour and 15 minutes; and with high heat, 45 minutes. At the end of each heating period, the acid was transferred to a 500-cubic-centimeter volumetric flask, made to volume, and the acid content determined as before. Ten determinations were made for each heating time to overcome any minor differences in the heat of the burners. The difference in quantity of acid present before and after heating was considered as the quantity volatilized by heating. Tests of acid losses were made, using the Kjeldahl [5] mixture with sodium sulphate instead of potassium sulphate. Tests were also made using the Gunning [6] mixture, and the mixture recommended by the Kansas City Protein Referee Board.[7]

To determine the acid loss due to oxidation of the wheat samples, 1-gram portions of wheat, varying in protein content from 8.09 to 18.70 per cent, were digested at medium heat for 75 minutes (complete oxidation) with the Kjeldahl mix (10 K_2SO_4–0.7 HgO). At the end of this period, the acid remaining was transferred to 500-cubic-centimeter volumetric flasks and the acid content determined as before. Subtracting this value from the acid remaining after heating gave the acid loss due to the digestion of the 1-gram sample.

The experiment was repeated with three samples using 25 cubic centimeters of acid and 2 grams of sample, measuring the acid content of each flask before and after heating, and after heating with the samples.

The data from these investigations are given in Table 2. They show that approximately 2 to 3.5 cubic centimeters of acid were lost by heating the acid together with the catalytic reagents. The smallest loss was obtained at the lowest intensity of heat and the loss increased as the heat was increased.

No greater heat appears to be developed by any one of the three formulas as measured by subsequent acid loss.

The acid loss owing to oxidation of the samples, when a 1-gram sample was digested, varied from 3.1 to 4.5 cubic centimeters. The average loss was 3.6 cubic centimeters. The total acid loss owing to heating of a medium nature and oxidation of a 1-gram sample was 6 cubic centimeters. Not quite as much, 5.6 cubic centimeters, were lost when the heat intensity was low, whereas 7.1 cubic centimeters were lost when a high heat was used. The total loss owing to heating of a medium nature and oxidation of a 2-gram sample was 9.8 cubic centimeters.

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[5] See footnote 1. [6] See footnote 2. [7] See footnote 3.

TABLE 2.—*Sulphuric acid*[1] *losses due to heating and to oxidation of wheat samples*

Treatment No.	Treatment	Protein	Intensity of heat	Acid used	Acid lost by heating	Acid lost by oxidation	Total loss due to heat and oxidation
		Per cent		*C. c.*	*C. c.*	*C. c.*	*C. c.*
1	Acid only starting		No heat	20			
2	10 gr. K_2SO_4 and 0.7 gr. HgO		Low	20	2.0		
3	do		High	20	3.5		
4	do		Medium	20	2.4		
5	10 gr. Na_2SO_4 and 0.7 gr. HgO		do	20	2.3		
6	10 gr. Na_2SO_4 and 0.2 gr. $CuSO_4$		do	20	2.5		
7	7 gr. Na_2SO_4 and 0.1 gr. $CuSO_4$ and 0.5 gr. HgO.		do	20	2.5		
8	Treatment 4: 1 gr. 11998	10.66	do	20	2.4	3.8	6.2
9	Treatment 4: 1 gr. 12002	12.88	do	20	2.4	3.4	5.8
10	Treatment 4: 1 gr. 12003	15.45	do	20	2.4	3.4	5.8
11	Treatment 4: 1 gr. 12005	12.77	do	20	2.4	3.4	5.8
12	Treatment 4: 1 gr. 12006	14.53	do	20	2.4	3.1	5.5
13	Treatment 4: 1 gr. 12007	10.20	do	20	2.4	4.5	7.9
14	Treatment 4: 1 gr. 12376	8.26	do	20	2.4	3.6	6.0
15	Treatment 4: 1 gr. 12868	8.09	do	20	2.4	3.7	6.1
16	Treatment 4: 1 gr. 12878	17.73	do	20	2.4	3.5	5.9
17	Treatment 4: 1 gr. 12883	18.07	do	20	2.4	3.4	5.8
18	Treatment 4: 2 gr. 12002	12.88	do	25	3.0	6.7	9.7
19	Treatment 4: 2 gr. 12006	14.53	do	25	3.0	7.0	10.0
20	Treatment 4: 2 gr. 12376	8.26	do	25	3.0	6.6	9.6

[1] Specific gravity of acid, 1.82 at 25° C.

Average acid lost from heat and oxidation of a 1-gram sample with 20 cubic centimeters of acid, 6.0 cubic centimeters, of a 2-gram sample with 25 cubic centimeters of acid, 9.8 cubic centimeters.

Knowing the acid losses as given, the optimum quantity of acid to use can then be calculated if further information is at hand regarding the acid necessary to hold the ammonia nitrogen split off from the protein molecule. To determine this, a portion of the work of Paul and Berry[8] was repeated and the data are given in Table 3.

TABLE 3.—*Sulphuric acid necessary to hold the ammonia present during the digestion period*

Concentrated sulphuric acid in flask	Protein present	Difference
SERIES I	*Per cent*	*Per cent*
$(NH_4)_2SO_4$ as protein added	13.57	
30 c. c. H_2SO_4, 10 gr. K_2SO_4, 0.7 gr. yellow HgO	13.62	0.05
25 c. c. H_2SO_4, 10 gr. K_2SO_4, 0.7 gr. yellow HgO	13.57	
20 c. c. H_2SO_4, 10 gr. K_2SO_4, 0.7 gr. yellow HgO	13.57	
15 c. c. H_2SO_4, 10 gr. K_2SO_4, 0.7 gr. yellow HgO	13.57	
10 c. c. H_2SO_4, 10 gr. K_2SO_4, 0.7 gr. yellow HgO	13.62	.05
5 c. c. H_2SO_4, 10 gr. K_2SO_4, 0.7 gr. yellow HgO	3.42	10.15
SERIES II		
$(NH_4)_2SO_4$ as protein added	13.57	
30 c. c. H_2SO_4, 10 gr. Na_2SO_4, 0.7 gr. yellow HgO	13.57	
25 c. c. H_2SO_4, 10 gr. Na_2SO_4, 0.7 gr. yellow HgO	13.57	
20 c. c. H_2SO_4, 10 gr. Na_2SO_4, 0.7 gr. yellow HgO	13.57	
15 c. c. H_2SO_4, 10 gr. Na_2SO_4, 0.7 gr. yellow HgO	13.57	
10 c. c. H_2SO_4, 10 gr. Na_2SO_4, 0.7 gr. yellow HgO	13.62	.05
5 c. c. H_2SO_4, 10 gr. Na_2SO_4, 0.7 gr. yellow HgO	3.76	9.81

This experiment was made with both sodium and potassium sulphates as catalytic agents, as it was felt by some of the collaborating laboratory personnel that sodium sulphate induced greater acid losses

[8] PAUL, A. E. and BERRY, E. H. THE KJELDAHL NITROGEN METHOD AND ITS MODIFICATIONS. Jour. Assn. Off. Agr. Chem., vol. 5, No. 1, pp. 108–32. 1921.

than potassium sulphate. This experiment was carried on at the greatest intensity of heat (see p. 5) for one hour.

Data obtained from this study, shown in Table 3, fully substantiate the claims of Paul and Berry,[9] that it is necessary to have at least 10 cubic centimeters of acid present to hold all the ammonia.

Therefore, in consideration of the data as given in Tables 2 and 3, it is evident that at least 10 cubic centimeters of acid are necessary to hold the ammonia formed during the oxidation process at all intensities of heat. It is necessary to have 6 cubic centimeters of acid to care for heat and oxidation losses when 1-gram samples are digested at a medium intensity of heat, 5.8 cubic centimeters at low heat, and 7.3 cubic centimeters at high heat, making the total quantity of acid necessary at the three intensities of heat; 15.8 cubic centimeters at low heat, 16 cubic centimeters at medium heat, and 17.3 cubic centimeters at high heat. When a 2-gram sample is used, 4 cubic centimeters additional acid must be used.

These acid losses will be subject to slight variations, depending upon the draft in the fume tubes or hood. Strong drafts will add about 1 cubic centimeter to the total given. A strong draft reduces the oxidation time approximately 10 per cent.

Under the heat and time conditions set forth in the preceding discussion, it is evident that 20 cubic centimeters of sulphuric acid are sufficient to digest completely a 1-gram sample of wheat and that 25 cubic centimeters of acid are sufficient to digest completely a 2-gram sample of wheat.

Further observations were made noting the effect of 15, 20, 25, and 30 cubic centimeters of concentrated acid upon the rapidity of oxidation of 1-gram samples. Using 15 cubic centimeters of acid at the greatest intensity of heat, too much frothing results. With 20 cubic centimeters of acid the digest cleared in 6½ minutes; with 25 cubic centimeters, in 8½ minutes; and with 30 cubic centimeters in 13 minutes. Thirty cubic centimeters of acid are too much at a high intensity of heat, as there is considerable bumping and spurting during the digestion process, which, no doubt, results in material losses. Protein determinations made at the end of 15 minutes, or before oxidation was complete, showed that oxidation had progressed further with 20 cubic centimeters than with any of the other quantities of acid used. It is reasonable to suppose that if this takes place at high heat it will also take place at the lower intensities of heat.

EFFICIENCY OF HEAT AND CATALYTIC AGENTS ON OXIDATION OF WHEAT SAMPLES WITH SULPHURIC ACID

As was pointed out, the periods of digestion and the catalytic agents were numerous within the formulas used by the various collaborators. No standard time or quantity of reagent was employed by the majority. The question arose as to the efficiency of heat and of these reagents in varying quantities as an aid to the rapid digestion of wheat samples.

The influence of heat on the oxidation process was studied by digesting wheats of high and low protein content for 30, 60, and 90 minutes with 20 cubic centimeters of concentrated sulphuric acid, at

[9] See footnote 8.

high, low, and medium intensities of heat. Data from this study are given in Table 4.

These data show that heat is the most important factor to be considered in digesting wheat samples. As great an oxidation can be obtained in 30 minutes with a high degree of heat as can be obtained in 90 minutes at low heat.

At all intensities of heat with the two samples tested, over 90 per cent of the protein nitrogen was in the form of ammonia within 30 minutes. In 90 minutes 94.25 per cent of the protein nitrogen was in this form at high heat, 93.2 per cent at medium heat, and 92.6 per cent at low heat.

These data also indicate that samples of low protein content digest at a slower rate than those of high protein content.

By increasing the heat 50 per cent above the maximum used in the above discussion it was found that over 99 per cent of the protein nitrogen in samples of wheat could be transformed into ammonia in less than 15 minutes. The flasks at this temperature were too hot to handle.

TABLE 4.—*Influence of heat and acid on protein test results*

Intensity of heat	Sample No.	Time					
		30 minutes		60 minutes		90 minutes	
		Protein found	Digested	Protein found	Digested	Protein found	Digested
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
High	6	14.03	91.55	14.42	93.76	14.53	94.47
Do	8	10.49	92.02	10.66	93.51	10.72	94.04
Medium	6	14.02	91.15	14.31	93.04	14.36	93.37
Do	8	10.26	90.00	10.49	92.02	10.60	92.98
Low	6	13.91	90.44	14.19	92.33	14.26	92.78
Do	8	10.20	89.47	10.36	90.87	10.54	92.45

In studying the effect of catalytic agents on the oxidation process, a shorter period of time was chosen than was necessary to complete the oxidation of the wheat samples, so that the heat factor would not mask the effects of the reagent studied. Two samples of wheat were used, one of high, the other of low protein content, and the results from each sample were averaged. The studies were made at low and medium intensities of heat. A 1-gram sample and 20 cubic centimeters of concentrated acid were used throughout, the catalyst being changed to suit the occasion. These data are shown in Table 5. In this table a value of 100 has been given to the results when the sample was digested with acid alone and when catalysts were used the results are expressed in terms of this value.

These data show that there is no advantage in choosing between red and yellow oxide of mercury in any quantity. The best quantity to use seems to be 0.5 gram. No advantage is gained by doubling the quantity of these reagents. Mercuric sulphate as a catalyst gave slightly lower results.

TABLE 5.—*The value of catalytic agents on protein test results*

Grams used	Mercury salts			K_2SO_4 and Na_2SO_4			$CuSO_4$ [1]	
	Yellow HgO	Red HgO	$HgSO_4$ [4]	Grams used	K_2SO_4	Na_2SO_4	Grams used	$CuSO_4$
	Per cent	*Per cent*	*Per cent*		*Per cent*	*Per cent*		*Per cent*
0.0	100	100	100	0	100	100	0.0	100
.1	102	103	102	3	103	103	.1	100
.3	104	104	103	5	104	103	.3	101
.5	105	105	104	7	104	104	.5	102
.7	105	105	104	10	105	104	1.0	101
1.0	105	105	104	15	105	105	2.0	100
							3.0	100

[1] Low intensity of heat only.

At low heat copper sulphate has no marked value as a catalyst. In fact, large quantities of this reagent reduced the rapidity of oxidation. At higher intensities of heat, however, this catalyst seems to work well.

Ten grams seems to be the best weight to use of either sodium or potassium sulphate, neither having any chemical advantage over the other. In choosing between these two salts as catalytic agents, the tendency of sodium sulphate to form an acid cake with sulphuric acid must be considered, along with the cost of these two reagents. Unless sodium sulphate digests are dissolved in water soon after cooling, time will be lost because of the difficulty of getting the salt cake into solution. To overcome this, it was found that a mixture of 40 per cent sodium sulphate and 60 per cent potassium sulphate will not form an acid cake.

Objection might be raised that a short period of time, such as 30 minutes, would not be sufficient for the catalysts to exert their full effect. To meet this objection, the heating time was extended to one hour with two of the catalysts.

From the data given in Table 6 it is seen that the effect of the catalyst was such that it reached its level immediately and remained there during the period of the test. From this it can be concluded that the full effect of catalytic agents is almost immediate and that further breaking up of the protein molecule is due to extended heating.

TABLE 6.—*Influence of time on activities of catalytic agents*

	Time of digestion			
	30 minutes		60 minutes	
	Nitrogen as c.c. N/10 alkali	Difference c.c. N/10 alkali	Nitrogen as c.c. N/10 alkali	Difference c.c. N/10 alkali
Red HgO:				
0.0 grams	17.25		17.89	
0.30 grams	17.92	0.67	18.59	0.70
0.70 grams	18.02	.77	18.69	.80
1.00 grams	17.97	.72	18.67	.78
Yellow HgO:				
0.0 grams	17.25		17.89	
0.30 grams	17.92	0.67	18.58	.69
0.70 grams	18.04	.79	18.58	.69
1.00 grams	18.04	.79	18.67	.78

INFLUENCE OF SIZE OF SAMPLE DIGESTED ON PROTEIN-TEST RESULTS

As 1 and 2 gram samples were used in this study, tests were made to note the influence of size of sample on results. Both 1 and 2 gram portions were digested for a time sufficient to afford complete oxidation. Samples were chosen having protein contents of from 8 to 18 per cent in 1 per cent ranges. The data are given in Table 7.

TABLE 7.—*Influence of size of sample digested on protein test results*

Test No.	Ranges in percentage of protein						
	10 to 10.99	11 to 11.99	12 to 12.99	13 to 13.99	14 to 14.99	15 to 15.99	16 to 20
One-gram sample:							
1	10.26	11.34	12.71	13.00	14.65	15.39	19.55
2	10.60	11.00	12.77	13.11	14.19	15.10	18.81
3	10.15	11.80	12.77	13.39	14.02	14.90	18.29
4	10.66	11.97	12.90	13.45	14.60	14.90	17.90
5	10.32	11.34	12.03	13.79	14.31	----------	----------
6	----------	11.97	12.60	13.51	14.82	----------	----------
7	----------	----------	12.54	13.05	----------	----------	----------
8	----------	----------	12.37	13.39	----------	----------	----------
Average	10.39	11.57	12.58	13.34	14.43	15.07	18.64
Two-gram sample:							
1	10.22	11.34	12.75	13.08	14.71	15.45	19.55
2	10.60	10.94	12.77	13.12	14.25	15.28	18.81
3	10.15	11.75	12.78	13.39	14.08	15.03	17.95
4	10.66	11.91	12.94	13.40	14.65	15.10	18.34
5	10.32	11.34	12.00	13.85	14.31	----------	----------
6	----------	11.97	12.60	13.62	14.83	----------	----------
7	----------	----------	12.54	13.11	----------	----------	----------
8	----------	----------	12.40	13.45	----------	----------	----------
Average	10.39	11.54	12.59	13.37	14.47	15.21	18.66
Difference of average	[1] 0.00	−0.03	+0.01	+0.03	+0.04	+0.14	+0.02

[1] Minus sign indicates a less quantity in the 2-gram sample.

The data show, on the average, that there is a slight advantage in using a 2-gram sample, especially with wheats containing the higher percentages of protein. This advantage is not sufficiently great to warrant its use, as it takes approximately 30 per cent longer to digest a 2-gram sample completely. When this factor is considered in the light of a laboratory making several hundred tests a day, the total additional time necessary to oxidize 2-gram samples will be considerable.

INFLUENCE OF METHOD, TIME, AND SAMPLE ON PROTEIN-TEST RESULTS

The time of digestion given to the samples by the various collaborators varied from 25 to 180 minutes. Less time was consumed by those using electricity as a source of heat than by those using gas. Three distinct methods were used: The Kjeldahl method, the Gunning method, and the Kansas City Protein Referee Board method. Large variations were also obtained by many laboratories with some of the spring wheats. Experiments were planned, therefore, to study the relation of these variables upon the protein test results. In this study the three different intensities of heat, described previously (see p. 5) were used. Three different methods

were used, with wheat having a high, medium, and low protein content. Samples from the hard red spring crop were chosen, as greater difficulty was experienced in obtaining uniform results from these samples. All samples were ground to pass through a No. 36 grits-gauze sieve. As shown in Table 8, the time was varied from 15 minutes to 2 hours in 15-minute intervals. Tests were run in triplicate and a blank determination was run as every tenth determination.

The digesting mixtures were as follows:

Kjeldahl mixture, 20 cubic centimeters acid, 10 grams K_2SO_4, 0.7 gram yellow HgO.

Gunning mixture, 20 cubic centimeters acid, 10 grams Na_2SO_4, 0.2 gram copper sulphate.

Kansas City Protein Referee Board mixture, 20 cubic centimeters acid, 7 grams Na_2SO_4, 0.5 gram $HgSO_4$, and 0.1 gram $CuSO_4$.

With the 1-gram sample, 20 cubic centimeters of acid was used throughout for the reasons given in the previous section.

Referring now to Table 8, it was found at low heat that the Kjeldahl method was the only one which digested the samples completely in less than one hour. With this intensity of heat, the Gunning method was very inefficient. In fact, complete oxidation was not obtained in two hours with this method. It took 75 minutes to digest the sample with the Kansas City Protein Referee Board method, whereas only an hour was required with the Kjeldahl method.

TABLE 8.—*Influence of time, method, and sample on protein test results*

KJELDAHL METHOD

Sample No.	Time of digestion—minutes							
	15	30	45	60	75	90	105	120
High heat:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
12004	12.77	12.79	12.85	12.86	12.88	12.88	12.88	12.88
12006	15.28	15.33	15.43	15.45	15.39	15.40	15.40	15.40
12008	11.23	11.37	11.40	11.41	11.40	11.40	11.42	11.43
Medium heat:								
12004	12.65	12.71	12.77	12.88	12.87	12.87	12.87	12.87
12006	15.10	15.22	15.28	15.43	15.42	15.43	15.44	15.42
12008	11.17	11.29	11.39	11.39	11.39	11.39	11.40	11.40
Low heat:								
12004	12.61	12.72	12.84	12.88	12.88	12.80	12.88	12.88
12006	15.12	15.18	15.29	15.45	15.45	15.45	15.39	15.45
12008	(1)	11.29	11.34	11.40	11.42	11.44	11.43	11.40

KANSAS CITY PROTEIN REFEREE BOARD METHOD

High heat:								
12004	12.65	12.70	12.82	12.82	12.82	12.82	12.82	12.82
12006	15.20	15.38	15.38	15.35	15.38	15.38	15.35	15.38
12008	11.17	11.37	11.39	11.40	11.44	11.44	11.42	11.40
Medium heat:								
12004	12.48	12.65	12.82	12.87	12.88	12.82	12.85	12–82
12006	15.22	15.38	15.38	15.39	15.38	15.38	15.38	15.38
12008	(1)	11.29	11.34	11.46	11.41	11.42	11.44	11.40
Low heat:								
12004	(1)	12.48	12.54	12.77	12.85	12.82	12.82	12.82
12006	(1)	15.04	14.98	15.15	15.38	15.33	15.38	15.40
12008	(1)	11.19	11.23	11.29	11.44	11.40	11.40	11.43

[1] Not clear. (See footnotes, p. 6.)

TABLE 8.—*Influence of time, method, and sample on protein test results*—Con.

GUNNING METHOD

Sample No.	Time of digestion—minutes							
	15	30	45	60	75	90	105	120
High heat:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
12004	12.37	12.74	12.84	12.82	12.83	12.87	12.81	12.85
12006	14.88	15.10	15.32	15.36	15.38	15.36	15.38	15.38
12008	11.00	11.34	11.40	11.39	11.39	11.44	11.41	11.40
Medium heat:								
12004	11.86	12.71	12.71	12.88	12.82	12.86	12.85	12.88
12006	14.59	15.10	15.22	15.38	15.38	15.38	15.38	15.38
12008	10.89	11.29	11.34	11.40	11.40	11.44	11.43	11.42
Low heat:								
12004	(1)	(1)	12.48	12.54	12.48	12.48	12.71	12.77
12006	(1)	(1)	14.65	15.03	15.16	15.16	15.22	15.22
12008	(1)	(1)	11.23	11.06	11.17	11.23	11.28	11.34

At medium heat, the Gunning method becomes more satisfactory and is in line with the other two methods. The digestion time with the Kansas City Protein Referee Board method was reduced 20 per cent by raising the heat from low to medium, whereas the time was reduced 50 per cent when the Gunning method was used. By using high heat it was found that all three methods were on a par. It was further brought out, when using high heat with a 1-gram sample and 20 cubic centimeters of acid, that no longer than 45 minutes is necessary to oxidize the sample completely.

A summary of the investigations in this section shows that protein tests results will vary, depending upon the length of time of digestion, and that this period of digestion depends upon the intensity of the heat. At high heat, by all three methods, complete oxidation can be obtained in 45 minutes; at medium heat, in 60 minutes. At low heat, the Kjeldahl method was the only one giving correct results in less than an hour. With the Kansas City Protein Referee Board method 75 minutes were necessary. The Gunning method was not satisfactory at this intensity of heat.

To check further the times recorded above, 19 samples representing the five commercial classes of wheat were digested for two hours. The same samples were also digested for 45 minutes at high heat and for 60 minutes at medium heat. As it is believed that low heat will never find place in commercial protein testing, it was not tried out. In the aggregate, with this intensity of heat the results are unsatisfactory. The oxidation is not regular and duplicative results are not the rule. The data from the check series, proving that 45 minutes is sufficient to oxidize a 1-gram sample at high heat and that 60 minutes is sufficient to do the same at medium heat, are given in Table 9.

TABLE 9.—*Protein content of 19 samples of wheat run by three methods at two different intervals of heat and time*

Sample No.	Series 1				Series 2			
	High heat, 45 minutes			Two hours Kjeldahl method	Medium heat, 60 minutes			Two hours Kjeldahl method
	Kjeldahl method	K. C. P. R. B. method	Gunning method		Kjeldahl method	K. C. P. R. B. method	Gunning method	
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	11.86	11.91	11.91	11.86	11.91	11.91	11.91	11.86
2	12.88	12.94	12.88	12.88	12.88	12.88	12.88	12.88
3	10.20	10.26	10.26	10.20	10.20	10.20	10.26	10.20
4	11.00	11.06	11.06	11.06	11.11	11.06	11.06	11.06
5	13.11	13.17	13.17	13.11	13.18	13.11	13.11	13.11
6	12.31	12.37	12.37	12.37	12.37	12.25	12.31	12.37
7	13.22	13.17	13.11	13.08	13.11	13.11	13.11	13.08
8	13.22	13.24	13.22	13.24	13.24	13.22	13.21	13.24
9	7.92	7.86	7.92	7.87	7.87	7.98	7.87	7.87
10	12.82	12.82	12.82	12.82	12.88	12.77	12.82	12.82
11	10.54	10.54	10.54	10.54	10.60	10.54	10.60	10.54
12	8.26	8.26	8.21	8.25	8.26	8.21	8.21	8.25
13	9.92	9.92	9.92	9.97	9.97	9.97	9.92	9.97
14	12.82	12.82	12.82	12.82	12.81	12.82	12.83	12.82
15	15.30	15.30	15.36	15.38	15.33	15.39	15.33	15.38
16	11.41	11.43	11.40	11.40	11.41	11.41	11.41	11.40
17	10.77	10.72	10.66	10.66	10.66	10.66	10.72	10.66
18	12.88	12.88	12.88	12.82	12.88	12.88	12.88	12.82
19	15.36	15.40	15.40	15.43	15.40	15.45	15.40	15.43

These figures are given for very carefully controlled heat conditions. It was pointed out earlier that the heat during the day and on different days was regulated by means of a gas-pressure regulator. This is not the case with most laboratories. It is a common observation to note changes in flow of gas as more or fewer burners are used. This, of course, will change the flow of heat to each individual flask, depending on the number in operation and the pressure in the gas mains during the day. It is felt, therefore, that in recommending 45 minutes at high heat, the variables mentioned will be eliminated, as in many cases almost complete oxidation was obtained at 30 minutes. The same is true for the data at medium heat, using 60 minutes.

Using 2 grams of sample, it will take 60 minutes to complete digestion at high heat and 75 minutes at medium heat. This is approximately 30 per cent longer time than was necessary with a 1-gram sample.

SAMPLING STUDIES

EFFECT OF DUPLICATE CAR SAMPLING ON PROTEIN-TEST RESULTS

It is absolutely essential that a car or lot of wheat be correctly sampled before proceeding with any test. Questions as to how close duplicate samplings of bulk loadings will agree in protein content have been frequently raised. For the purpose of obtaining some data on this point, duplicate samplings were made of 241 cars of wheat as they arrived at the Minneapolis market. Some of these cars contained as high as 15 per cent of dockage and represented receipts loaded both evenly and unevenly. Each car was sampled twice in five different areas by means of the usual grain probe in

the manner illustrated in Figure 3. The contents of probes 1 to 5 and 6 to 10, inclusive, were composited separately, thoroughly cleaned, mixed, and reduced to 75 grams in size to be ground for making protein tests. The mixing and reducing process were accomplished by the means of a Boerner sampler. The results of these tests are shown in Table 10. Moisture determinations were made at the time the protein determinations were made so that the results reported are on a uniform moisture basis.

TABLE 10.—*Variation in protein results obtained from analysis of duplicate samplings of 241 car lots of spring wheat*

[Basis 13.5 per cent moisture]

Number of cars sampled		85	56	53	27
Percentage variation in duplicate tests		0 to 0.04	0.05 to 0.09	0.10 to 0.14	0.15 to 0.19
Percentage of total car samplings		35.2	23.2	22.0	11.2
Number of cars sampled	10	2	4	2	2
Percentage variation in duplicate tests	0.20 to 0.24	0.25 to 0.29	0.30 to 0.34	0.35 to 0.39	0.40
Percentage of total car samplings	4.2	.8	1.7	.8	.8

Data from these tests show that over 96 per cent of the test varied by less than 0.25 per cent. This value, moreover, is the variation

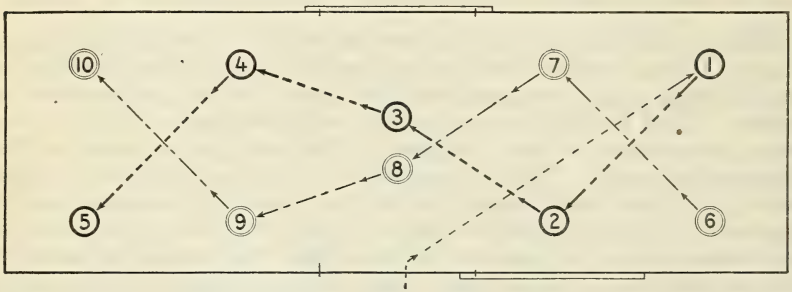

FIG. 3.—Showing method used in sampling of wheat for the duplicate sampling test described in the text. Arrows show the sequence of sampling

permitted by the American Association of Cereal Chemists [10] in the matter of making protein tests. It is evident then that car lots of wheat can be sampled a number of times with a reasonable degree of accuracy, providing, of course, that other conditions effecting the protein tests be controlled.

CLEANING SAMPLES BEFORE ANALYSIS

The methods used in preparing the samples for protein analysis, especially the removal from the sample of foreign material, show need for improvement, as they are far from uniform. Sieves, dockage machines, and forceps are regularly used to remove foreign material. As the result of using oversized sieves, cases are known in which small-sized kernels, high in protein, are regularly sieved out, making concordant tests difficult.

[10] MANGELS, C. E., and others. REPORT OF COMMITTEE ON METHODS. Jour. Cereal Chem., vol. 2, No. 4, p. 235.

TAKING OUT THE SUBSAMPLE

Before a subsample is taken, the original bulk should be thoroughly mixed because of the influence which sizes and types of wheat kernel have on the protein test results. It is common knowledge that the dark, hard, and vitreous kernels of wheat in any given sample have more protein than the soft and chalky kernels. This has been brought out by Frank [11] and is supported by the data in Table 11. Differences in protein content of dark, hard, and vitreous kernels as compared to chalky kernels will average about 1.5 per cent and cases are on record in which the differences were as great as 4 per cent. If the quantities of dark, hard, and vitreous and chalky kernels vary appreciably, differences in results are bound to occur unless the samples are thoroughly mixed and reduced by use of a sampling device. Therefore one can not be too careful in getting a representative sample of the grain to be analyzed before making the tests.

TABLE 11.—*Protein content of yellow and chalky and dark, hard, and vitreous portions of hard red winter and hard red spring wheats*

	Hard red winter wheats		Hard red spring wheats	
	Dark, hard, and vitreous	Chalky	Dark, hard, and vitreous	Chalky
Number of samples	156	156	70	70
Average protein content—per cent	12.34	11.11	13.15	11.42
Maximum protein content—do	16.24	15.21	16.24	14.31
Minimum protein content—do	8.82	7.40	11.58	9.74
Range in protein content—do	7.42	7.81	4.66	4.57

CORRECT SIZE OF SAMPLE TO GRIND

From the collaborative data it was found that the quantity of wheat ground varied from 6 to 150 grams. To determine how great an effect the grinding of small and large quantities would have on the protein test results, 1,000-gram portions of each of the 12 samples submitted for collaborative tests were mixed thoroughly and passed through the Boerner sampler and 8 to 15, 30, 60, and 125 gram portions were drawn off, ground, and protein tests made thereon.

The data given in part A of Table 12 record the protein percentages without taking into consideration the moisture content of the sample. As much as 3 per cent of moisture may be lost in the grinding process. Differences of as much as 0.4 per cent of protein were obtained from the results of grinding varying quantities of wheat. The greatest differences were obtained with samples of 8 and 15 grams.

[11] FRANK, W. L. PROTEIN CONTENT VS. TEXTURE VS. KERNEL WEIGHT IN SOUTHWESTERN MARKET-RUN WINTER WHEAT. Modern Miller, July 14, 1923, p. 23.

TABLE 12—*Influence of size of sample ground on protein test results*

PART A

(Results are on the basis of the moisture as present in sample)

Laboratory No.	Size of sample ground (grams)					Maximum difference
	8	15	30	60	125	
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
11998	10.94	10.60	10.77	10.72	10.66	0.34
11999	12.08	11.86	11.86	11.86	11.86	.22
12000	13.00	13.11	13.11	13.05	13.05	.11
12001	12.94	12.82	12.82	12.77	12.75	.19
12002	13.22	12.88	12.94	12.82	12.88	.40
12003	15.39	15.28	15.28	15.33	15.10	.29
12004	12.37	12.25	12.20	12.20	12.20	.17
12005	13.05	12.77	12.84	12.82	12.77	.28
12006	14.31	14.48	14.53	14.53	14.53	.22
12007	10.15	10.20	10.20	10.15	10.20	.05
12008	10.66	10.66	10.60	10.54	10.60	.12
12009	11.11	11.11	11.06	11.06	11.06	.05

PART B

(Results are on the basis of 13.5 per cent moisture)

11998	10.55	10.35	10.50	10.48	10.50	.20
11999	11.69	11.69	11.61	11.58	11.65	.11
12000	12.61	12.66	12.67	12.63	12.62	.06
12001	12.51	12.50	12.51	12.42	12.44	.09
12002	12.60	12.46	12.52	12.58	12.46	.14
12003	14.71	14.76	14.82	14.89	14.79	.18
12004	11.81	11.71	11.75	11.79	11.83	.12
12005	12.45	12.35	12.39	12.44	12.44	.10
12006	13.96	14.13	14.25	14.21	14.21	.29
12007	9.62	9.83	9.89	9.79	9.83	.27
12008	10.25	10.35	10.31	10.29	10.38	.13
12009	10.90	10.80	10.76	10.78	10.78	.14

From the data in part B of Table 12, it is seen that there were some differences in the protein test results due to size of sample ground, even when the moisture factor was considered. The smaller the size of the sample ground, the larger were these differences. That is to say, grinding only 8 to 15 grams of sample introduced an appreciable error even if moisture conditions were controlled. The differences in protein results when 30 grams or more were ground were very small, being in most cases well within the usual limit of error, 0.1 per cent. It is believed, however, that these grindings were carried out much more carefully than is the case in the average protein laboratory.

CARE OF GRINDING APPARATUS

At present most laboratories are equipped with small burr flouring mills. These give satisfactory grinding service. The only precaution to be pointed out is to be sure the burrs in the mill are cleaned thoroughly before additional samples are run through. This can be done by running through not less than 15 to 20 grams of the new sample before taking any of it for the test. Proof of this is given in Table 13. A sample of high protein wheat was ground until no further meal was obtained and the residue was left in the

mill. A second sample of low protein wheat, in 5, 10, 15, 20, and 25 gram portions, was then passed through and the protein test was made on each grinding. After the 25-gram portion was run through, the mill was cleaned and a second 25-gram portion of the sample was cleaned with 5, 10, 15, 20, and 25 gram portions of the high protein wheat. The experiment was repeated with second high and low protein wheat.

It is evident that from 15 to 20 grams of wheat are sufficient to clear the mill for any new samples. A small quantity of high or low protein wheat left in the burrs will cause erroneous results.

TABLE 13.—*Grams of wheat necessary to clean small-burr mill in order to obtain correct results*

Treatment	Protein	Treatment	Protein
SERIES I	*Per cent*	SERIES III	*Per cent*
Sample 12003	15. 45	Sample 12007	10. 26
Cleaned with 5 grams 11998	12. 25	Cleaned with 5 grams 12006	13. 22
Cleaned with 10 grams 11998	10. 94	Cleaned with 10 grams 12006	14. 36
Cleaned with 15 grams 11998	10. 72	Cleaned with 15 grams 12006	14. 45
Cleaned with 20 grams 11998	10. 72	Cleaned with 20 grams 12006	14. 48
Cleaned with 25 grams 11998	10. 72	Cleaned with 25 grams 12006	14. 50
SERIES II		SERIES IV	
Sample 11998	10. 72	Sample 12006	14. 50
Cleaned with 5 grams 12003	13. 39	Cleaned with 5 grams 12007	11. 80
Cleaned with 10 grams 12003	15. 28	Cleaned with 10 grams 12007	10. 32
Cleaned with 15 grams 12003	15. 45	Cleaned with 15 grams 12007	10. 20
Cleaned with 20 grams 12003	15. 50	Cleaned with 20 grams 12007	10. 25
Cleaned with 25 grams 12003	15. 45	Cleaned with 25 grams 12007	10. 29

INFLUENCE OF FINENESS OF DIVISION OF SAMPLE ON PROTEIN-TEST RESULTS

The fact that little difficulty is experienced in getting concordant results when making protein determination on flour is evidence that fineness of division in grinding may have a bearing on the accuracy of protein tests in wheat.

To study this point, 100-gram portions of the same 12 samples of wheat before described were analyzed in five different states of division, ranging from the whole seed to that condition where 100 per cent of the ground sample passed through a No. 36 grits gauze. The fineness of division series in detail were: Whole kernel, kernels ground so that 15, 30, 60 and 100 per cent, respectively, passed through a No. 36 grits gauze. Before analysis, the samples were carefully mixed on paper to insure uniformity.

Data on this subject are given in Table 14. Part A records the analyses in which no consideration was given to the moisture content, and part B the data on a 13.5 per cent moisture basis.

It is evident from these tests that the fineness to which a sample is ground influences the accuracy of protein tests. This is doubly true when moisture is not considered. The maximum difference under such conditions, as shown in Table 14, part A, was found to be 0.45 per cent. The results with whole kernels are irregular. Sometimes they are high and at other times low, as compared with the results from carefully ground samples.

TABLE 14.—*Influence of fineness of division in grinding of sample on protein-test results*

PART A

[Results are on the basis of the moisture as present in sample]

Laboratory No.	99 per cent passing 36-grits gauze	60 per cent passing 36-grits gauze	30 per cent passing 36-grits gauze	15 per cent passing 36-grits gauze	Whole seed	Maximum difference
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
11998	10.60	10.77	10.66	10.52	10.32	0.45
11999	11.91	11.91	11.80	11.66	11.63	.28
12000	12.94	13.11	12.88	12.88	13.05	.23
12001	12.78	12.82	12.60	12.60	12.54	.28
12002	12.75	12.71	12.60	12.60	12.37	.38
12003	15.45	15.28	15.16	15.28	15.16	.29
12004	12.20	12.14	12.08	12.03	12.08	.17
12005	12.75	12.77	12.77	12.65	12.77	.12
12006	14.53	14.53	14.25	14.14	14.08	.45
12007	10.22	10.15	10.15	10.15	10.09	.13
12008	10.60	10.72	10.54	10.54	10.43	.29
12009	10.94	11.06	11.00	10.83	10.66	.40

PART B

[Results are on the basis of 13.5 per cent moisture.]

11998	10.47	10.53	10.51	10.52	10.26	0.27
11999	11.62	11.65	11.67	11.66	11.59	.08
12000	12.62	12.71	12.58	12.59	12.80	.22
12001	12.45	12.51	12.40	12.43	12.38	.13
12002	12.42	12.41	12.39	12.40	12.23	.19
12003	14.85	14.78	14.72	14.87	14.81	.15
12004	11.75	11.80	11.70	11.75	11.77	.10
12005	12.34	12.45	12.46	12.50	12.55	.21
12006	14.25	14.20	14.27	14.28	14.02	.26
12007	9.84	9.77	9.83	9.88	9.85	.11
12008	10.38	10.41	10.37	10.35	10.31	.10
12009	10.81	10.83	10.80	10.73	10.56	.27

Eliminating whole seed, it is surprising to find that such concordant results as are shown here can be obtained, regardless of the fineness of division of the sample. This is attributed to the care taken in mixing the sample after grinding, as preliminary trials on mill-run material gave very irregular results. Although nothing of significance developed (the data from whole kernel excepted) from the standpoint of fineness of division, the experiment brings out the point that if ground samples are thoroughly mixed after grinding, reliable analyses will result, even though the samples have not been ground to a fine state of division. It has been the experience and observation of the writers that unless the samples of the ground material are thoroughly mixed, incorrect results will occur. Moreover, grinding to a fine state of division eliminates the necessity for extensive mixing.

INFLUENCE OF MOISTURE CONTENT OF SAMPLE ON PROTEIN-TEST RESULTS

When samples are ground in an attrition mill in preparation for digesting, changes in moisture are bound to occur. How great this error will be depends upon such factors as size of sample ground, moistness of sample, how cool the mill remains with continued use, etc.

Experience in the chemical research laboratory of the grain division has been that the smaller the sample ground, the larger the moisture loss. In the experiment listed in Table 14, sample 11998 contained 10.34 per cent moisture when 8 grams were ground, and 11.41, 11.26, 11.56, and 12.20 per cent, respectively, when 15, 30, 60, and 125 grams were ground. Sample 12002 contained 9.25, 10.29, 10.63, 11.82 and 12.34 per cent moisture when the same quantities of seed were ground; and sample 12005 had 9.52, 10.73, 10.35, 10.85, and 11.42 per cent moisture under the same conditions.

Moist or wet wheat is not only difficult to grind but will lose a greater percentage of moisture during grinding than dry wheat. Questions have been frequently asked as to how great this loss is and how the protein results would differ if they were reported on a moisture test, such as the Brown-Duvel[12] test; also how other results would vary from the Brown-Duvel test if different methods of making moisture tests were used.

To obtain data on this point, 20 samples of wet wheat were obtained and moisture determinations made by the following methods: (1) By the Brown-Duvel method; (2) by heating the whole grain for 120 hours under atmospheric pressure at a temperature between 99 and 100° C.; (3) after grinding, by the vacuum-oven method for a period of five hours at a temperature of 99.5° C. and at a pressure of 4 inches of mercury; (4) after grinding, by the air-oven method under atmospheric pressure and at a temperature of 108° C. for a period of five hours; (5) after grinding, by the water-oven method, under atmospheric conditions at a temperature of 99.5° C. for a period of five hours.

By the Brown-Duvel method the average moisture content of the samples was 14.9 per cent. Drying the whole seed to constant weight in the water oven gave an average of 15.01 per cent moisture. On the ground seed by the vacuum-oven method the moisture content was 14.17 per cent; by the electric-oven method, 13.90 per cent; and by the water-oven method, 13.68 per cent. The maximum difference by all methods was 1.39 per cent. Some of this can be attributed to the loss of moisture due to grinding. Necessarily, if a uniform method is not used for making moisture tests, uniform protein results can not be obtained.

For each per cent change in the moisture content of the sample there will be a corresponding change, equivalent to 1 per cent of the protein content originally present. This change in protein content will be in the inverse order to the change in moisture content. That is to say, if a sample of wheat contains 12 per cent of protein at a moisture content of 13 per cent and dries to 12 per cent, the protein content on the new moisture basis will be 12.12 per cent. Tables for converting protein values at any given moisture content to a unit basis of 13.5 per cent moisture will be found in Miscellaneous Circular No. 28, United States Department of Agriculture.

Lack of a uniform method for making moisture tests is further illustrated by the moisture results obtained by 9 of the protein testing laboratories on the 12 samples of wheat submitted for protein tests. The data which are given in Table 15 show a range of 1.46

[12] See footnote 4.

per cent in the moisture content of the samples submitted, enough difference to change appreciably the protein content of a sample of wheat, especially one of high protein content.

DISTILLATION TECHNIQUE

Investigations show that the outstanding factors necessary to obtain reliable results from the distillation side of protein testing are: Use of traps of sufficient size to prevent spray from being carried over during distillation; more frequent "blank" determinations; use of standard solutions of such strength that careful measurements will enable the analyst to work efficiently; use of sufficient receiving acid to catch all the ammonia formed from samples having a large range in protein content; use of sufficient reagent to precipitate the mercury when this is used as a catalyst; and the accurate preparation of standard solutions.

TABLE 15.—*Moisture results obtained by various laboratories on two series of wheat samples submitted in sealed tin containers*

Laboratory identification	1	2	3	4	5	[1] 6	7	8	9	10	11	[2] 12
	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
1	13.04	13.28	11.51	12.33	12.27	10.49	11.04	11.45	13.35	10.23	13.78	12.70
2	12.75	13.10	11.40	12.20	11.95	10.10	11.02	11.41	13.17	10.13	13.57	12.54
3	13.34	13.77	11.86	12.75	12.56	10.58						
4							11.58	11.74	13.81	10.58	14.12	13.07
5	13.64	13.97	12.14	12.97	12.78	10.81						
6							11.26	10.85	12.66	10.37	12.96	12.03
7	12.37	12.73	11.10	11.85	11.45	9.88	9.94	10.40	12.40	9.42	12.98	12.01
8							10.18	10.66	12.44	9.50	12.66	11.80
9	13.05	13.20	11.41	12.26	12.13	10.29	10.95	11.15	13.50	10.50	13.82	12.65
Average	13.03	13.34	11.57	12.39	12.19	10.36	10.85	11.09	13.05	10.11	13.41	12.40
Maximum	13.64	13.97	12.14	12.97	12.78	10.81	11.58	11.74	13.81	10.58	14.12	13.07
Minimum	12.37	12.73	11.10	11.85	11.45	9.88	9.94	10.40	12.40	9.43	12.66	11.80
Range	1.27	1.24	1.04	1.12	1.33	.93	1.64	1.34	1.41	1.15	1.46	1.27

[1] 1 to 6, spring wheat.

[2] 7 to 12, winter wheat.

The experience of this laboratory has been an exact duplication of the findings of Paul and Berry.[13] On the subject of Kjeldahl traps, the necessity of using large double-lipped traps, whenever possible, to obtain the best results, especially when the distillation is carried on rapidly, needs to be emphasized. The use of traps of various sizes and shapes on the same still will lead to erroneous results.

Blank determinations should be run more frequently. Blanks are run by some of the collaborators only every week or every three or four days. Blanks run on the same reagents for several successive days have been known to vary from 0.1 to 0.2 cubic centimeters of 0.1N alkali. This in terms of protein is equal to 0.06 to 0.12 per cent.

As for the strength of receiving acids, it is believed that the use of half normal solutions should be eliminated as their use introduces too great a chance for error. For convenience, especially when using a 2-gram sample, 0.2 normal acid has given satisfaction. Solutions for back titrations should not be stronger than 0.1253 normal, preferably 0.1 normal.

As the usual wheat crop will have a protein content varying from 8 to 20 per cent, it is necessary to have present sufficient acid to hold

[13] See footnote 8.

35 milligrams of nitrogen if a 1-gram sample is digested, or 70 milligrams if a 2-gram sample is digested. These conditions would require 12.5 cubic centimeters of 0.2 normal acid when a 1-gram sample is digested, and 25 cubic centimeters when 2-gram samples are taken for analysis.

Again referring to the article by Paul and Berry,[14] it is found (1) that their figures for the volume of distillation are correct. According to the figures of this laboratory, namely, practically all the ammonia passes over with 75 cubic centimeters of the distillate and all of it with 100 cubic centimeters; and (2) when sufficient acid is present to neutralize 85 per cent of the distillate no loss of the uncombined ammonia takes place. The writers find it is advisable, however, to use 50 cubic centimeters of water in the receiving flask so that the nose of the glass tubing will be immersed.

CHOICE OF RECEIVING ACIDS

So far as receiving acids are concerned, there is apparently no difference between hydrochloric and sulphuric. It was thought by some that hydrochloric acid would not hold the ammonia distilled over at the high temperatures sometimes occurring in the distillation flasks. This is not the case, as can be seen by consulting Table 16.

TABLE 16.—*Influence of temperature on ability of HCL, H_2SO_4, and boric acids to hold ammonia*

Acid	Per cent NH_3 retained at a temperature of —							
	25° C.	30° C.	35° C.	40° C.	45° C.	50° C.	60° C.	70° C.
HCL	100	100	100	100.00	100.00	100.00	100.00	100.00
H_2SO_4	100	100	100	100.00	100.00	100.00	100.00	100.00
Boric	100	100	100	99.25	98.44	96.71	[1] 90.19	86.89

[1] Can smell NH_3 at this temperature.

This table records the data comparing the ability of HCL, H_2SO_4, and boric acid to hold ammonia at increased temperatures. For this study, 10 cubic centimeters of approximately 0.1 normal NH_4OH was added to 0.1 normal HCL and H_2SO_4, and 4 per cent boric acid solutions. The acid was present to the extent of 50 per cent more than was necessary to neutralize the ammonia. The temperature varied in 5° intervals from 25° to 50° C., then by 10° intervals to 70°. It will be seen that there was no loss of ammonia when hydrochloric or sulphuric acid was used, but that boric acid began to lose ammonia at 40° and the quantity lost gradually increased as the temperature was raised to 70°.

Boric acid will also release or lose ammonia on standing. Titrations were made after adding ammonia and allowing to stand for 1, 2, 3, 4, 5, 6, 7, 8, 16, and 24 hours. No ammonia was lost in 4 hours but at the end of 5 hours, 0.85 per cent of the original ammonia was lost and at 6, 7, 8, 16, and 24 hours, 0.86, 1.23, 1.99, 2.89, and 5.18 per cent of ammonia was lost at the intervals listed. Boric acid, there-

[14] See footnote 8.

fore, can be used with safety only when the temperature of the distillate is not greater than 35° C. It must also be titrated within 4 hours after the ammonia has been introduced. It is evidently not safe to allow it to stand overnight, contrary to the findings of Scales and Harrison.[15]

These data are also contrary to that recently submitted by Markley and Hann [16] who find no appreciable loss of ammonia at 50° C.

MERCURY PRECIPITANTS

The use of potassium and sodium sulphide seems to be general among collaborators using mercury as a catalytic agent. But four of the collaborators used sodium thiosulphate for this purpose. This reagent, providing it is as effective as the first two, has a decided advantage over the sulphides, as it is less expensive and has no repulsive odor. Tests were made of its efficiency as a mercury precipitant and it was found that 1 gram of sodium thiosulphate is sufficient to precipitate 0.9 gram of metallic mercury. It was also found, during the investigations, that this quantity of mercury will hold back approximately 13 per cent of the nitrogen present. This is close to the figures as given by Paul and Berry.[17] It is, therefore, suggested that this reagent be given larger use, especially where objection is made to the odor of the sulphides of potassium and sodium, as is often the case in office buildings.

STANDARD SOLUTIONS

To gather some information relative to the accuracy of standard solutions, 8 ounces of sulphuric acid were sent to each collaborator with the request that he standardize the solution and report its value. These figures are given in Table 17 and show that the normality as submitted by 34 collaborators varied from 0.13003 to 0.1256, or a difference of 0.00443. Expressed in terms of protein, this is equivalent to 0.35 per cent, a very appreciable amount when it is considered that the Kansas City Board of Trade requires a recheck analysis when the protein test varies more than 0.12 per cent. It is felt that special attention has been given to this standardization work and that the usual routine in this field would show even wider variables.

As an added interest, the same sample of acid was sent to the United States Bureau of Standards for test and the reported normality value is given under the caption X in Table 17. This value was obtained gravimetrically by weighing the sulphate as barium sulphate. This value was further checked volumetrically by titration with a standard alkali solution. The two values were identical. No nonvolatile sulphates were found.

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[15] SCALES, F. M., and HARRISON, A. P. BORIC ACID MODIFICATION OF THE KJELDAHL METHOD FOR CROP AND SOIL ANALYSIS. Jour. Ind. Eng. Chem., vol. 12, No. 4, p. 350. 1920.

[16] MARKLEY, K. S., and HANN, R. M. A COMPARATIVE STUDY OF THE GUNNING-ARNOLD AND WINKLER BORIC ACID MODIFICATIONS OF THE KJELDAHL METHOD FOR THE DETERMINATION OF NITROGEN. Jour. Assoc. Off. Agri. Chemists, No. 4, vol. 8, pp. 455–467. 1925.

[17] See footnote 8.

TABLE 17—*Normality figures given to a sample of sulphuric acid by 34 collaborators*

Number of collaborator	Normality submitted	Number of collaborator	Normality submitted	Number of collaborator	Normality submitted	Number of collaborator	Normality submitted
1	0.1276	10	.1274	20	0.1268	34	0.12826
2	.1286	11	.1276	21	.1274	36	.1288
3	.1290	12	.1282	22	.12735	37	.1268
4	.1268	13	.1288	23	.1277	39	.1295
5	.1297	14	.12775	24	.1288	42	.1276
6	.1276	15	.12852	27	.1282	45	.1256
7	.1290	16	.1294	29	.1286	X [1]	.12974
8	.1285	17	.1296	31	.13033		
9	.1286	18	.1280	33	.12771		

[1] United States Bureau of Standards.

Average normality found	0.12823	Range in normality found	0.00443
Maximum normality found	.13003	Protein equivalent, per cent	.35
Minimum normality found	.12560		

The results obtained by 34 of the collaborators show that a decided effort should be made to standardize the manner of determining the values of normal solutions. Eight distinct reagents were used to determine the normality of alkali solutions, namely, benzoic acid, constant boiling point hydrochloric acid, hyrochloric and sulphuric acid solutions of known normality, oxalic acid, potassium-acid-phthalate, potassium bitartrate, and succinic acid. In many cases the protein results are calculated direct from the values of the standard alkali. In other instances, however, a cross titration is made and the results are calculated on the value of standard acid. Such a practice usually results in different normality values, especially when compared with results obtained by precipitation and weighing of either hydrochloric or sulphuric acid.

The Bureau of Standards in submitting their results stated in connection with standardizing solutions volumetrically:

> The limit of accuracy of volumetric analysis is about 1 part in 500 unless special precautions are taken. All apparatus used at this bureau is calibrated here and the tolerance permitted in the calibration will permit errors of this magnitude. Uncalibrated apparatus has been found to give errors very much in excess of this ratio.
>
> A solution standardized at 20° C. and used in correctly calibrated burettes will be in error 1 part in 1,000 if used at 25° C. and no account is taken of the expansion of the solution due to rise in temperature.
>
> The accuracy in reading the burette exerts another limiting effect upon the accuracy of the result as well as the ability of the analyst to bring the solutions titrated to the same degree of neutrality. When all of these conditions are considered, accuracies of 1 part in 500 are considered satisfactory and the expression of results to a greater degree of accuracy has no significance.

It is suggested that careful consideration be given to standardizing solutions with potassium acid phthalate. This salt can now be obtained in very pure form, is nonhygroscopic, and can be very accurately weighed on account of its high molecular weight. Furthermore, the end point in titration with sodium or potassium hydroxide is remarkably clean cut. The use of this salt as a standard in volumetric analysis has been fully discussed by Hendrixon [18] and

[18] HENDRIXSON, W. S. ACID POTASSIUM AND ACID SODIUM PHTHALATES AS STANDARDS IN ACIDIMETRY AND ALKALIMETRY. Jour. Amer. Chem. Soc., vol. 37, p. 2352.

by Dodge[19] and their data is well worth studying. The use of potassium acid phthalate has been entirely satisfactory in this laboratory.

METHOD RECOMMENDED FOR MAKING PROTEIN TESTS

PROCURING THE SAMPLE

The procuring of a representative sample is essential to the determination of the true value of a given lot of grain. If the sample obtained is not representative, no amount of care in making determinations will establish the protein content of the grain samples. Consequently, great care should be taken in sampling and the recommendations on the subject given in the Handbook of Official Grain Standards, Form 90, issued by the United States Department of Agriculture carefully considered.

CLEANING THE SAMPLE

Wheat samples should not be analyzed for protein content until the dockage has been removed from the sample. Dockage includes sand, dirt, weed seeds, weed stems, chaff, straw, grain other than wheat, and any other foreign material which can be removed readily from the wheat by the use of appropriate sieves, cleaning devices, or other practical means suited to separate the foreign material present. It also includes undeveloped, shriveled, and small pieces of wheat kernels which can be removed in separating the foreign material, and which can not be recovered by properly rescreening or recleaning.

REMOVING THE DOCKAGE

Such sieve or sieves should be used as will remove the foreign material with the least possible loss of wheat, including small, plump, or badly shriveled kernels, or large pieces of broken kernels.

The details for the method for removing dockage are given in United States grain standards act, Handbook of Official Grain Standards, form 90, issued by the United States Department of Agriculture.

DIVIDING SAMPLE FOR TEST

After a representative sample of the lot or parcel of grain is obtained, it is usually necessary to reduce its size considerably. To reduce the size of a sample of grain containing foreign substances of different specific gravity or size than of the grain with which they are mixed, and at the same time obtain a sample as representative as the original, is hardly possible except by mechanical means.

The device, generally referred to as the Boerner sampler, which will divide a sample into small portions and still maintain the proper proportions for the various factors of the original sample, is the best for this purpose. The device is fully described in United States Department of Agriculture Bulletins Nos. 287 and 857.

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[19] DODGE, F. D. THE STANDARDIZATION OF ALKALIMETRIC SOLUTIONS. Jour. Ind. Chem., vol. 7, p. 29. 1915.

GRINDING SAMPLE PREPARATORY TO CHEMICAL ANALYSIS

Thirty to forty grams of wheat should be ground on a burr mill to such a degree of fineness that at least 60 per cent of it will pass through a No. 36 grits-gauze sieve. The burr mill should always be kept cool. The ground material should be immediately placed in a suitable air-tight container. The container should be of such size as to allow intimate mixing of the sample.

The grinding apparatus should be thoroughly cleaned before each new sample of wheat is ground. This may be accomplished by disassembling the mill or by passing a sufficient quantity of the new sample through the burrs so that all traces of the previous sample will be entirely removed.

CHEMICAL REAGENTS NECESSAY FOR MAKING PROTEIN TEST

As a receiving acid for ordinary work, N/8 or N/10 acid is recommended. For standardizing hydrochloric and sulphuric acid the following methods are recommended.

(*a*) *Standard hydrochloric acid.*—Determine the absolute strength as follows: Preliminary test: Place a measured portion of the acid to be standardized in an Erlenmeyer flask with excess of calcium carbonate to neutralize free acid and a few drops of potassium chromate as indicator by titration with silver nitrate solution determine exactly the quantity required to precipitate the chlorides. Final determination: To a measured portion of the acid to be standardized add from the burette 1 drop in excess of the required quantity of silver nitrate solution as determined by the preceeding test. Heat to boiling, protect from the light, and allow to stand until the precipitate is granular. Filter on a tared Gooch crucible, previously heated to 140–150° C., cool and weigh.

(*b*) *Standard hydrochloric acid* (*constant boiling point acid*)[20].—Make up HCL to a density of approximately 1.1, using a hydrometer. Distill off about three-fourths of this from an ordinary distillation flask, and discard the distillate. Continue the distillation of the remaining one-fourth, collecting this distillate in a separate container. This is the "constant boiling" acid, having a very definite composition which varies but slightly with variations in the atmospheric pressure at which the distillation is carried out. The desired quantity of this acid is weighed out in an open dish on a sensitive balance. The open dish may be used since the acid is already in equilibrium with the air.

The following tabulation shows the composition of constant boiling acid at various pressures, as well as the corresponding quantities necessary to give 1 gram-molecule of HCL. If this quantity is diluted to 1 liter, an exactly normal solution of HCL results. If diluted to 10 liters exactly N/10 HCL results.

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[20] Jour. Amer. Chem. Soc. vol. 31, p. 390.

Pressure	Per cent HCL	Gram distillate for 1 mol of HCL
770	20.218	180.39
760	20.242	180.17
750	20.266	179.96
740	20.290	179.74
730	20.314	179.53
720	20.338	179.31

For correcting weights to vacuum (brass weights), add 0.17 gram for each mol of HCL.

If no barometer is available, the barometric pressure may be obtained by calling up the local weather office. It is to be noted, however, that an error of several millimeters would effect no very appreciable error in the final solution.

Only pure CO_2-free distilled water should be used for diluting the constant boiling acid to the desired strength. The water need not be boiled, but may be satisfactorily purified by aspirating it with air which first passes through soda-lime, until 1 drop of N/10 NaOH added to 200 cubic centimeters produces a distinct pink color in the presence of a few drops of phenolphthalein.

If these directions are all carefully followed one can not fail to obtain exact solutions, providing, of course, that the weights and measuring apparatus are accurate. The method is not only extremely accurate, but requires relatively little time and attention and does away with the necessity of relying upon purity of reagents.

(*c*) *Standard Sulphuric Acid.*—Determine the absolute strength of the acid by precipitation with barium chloride solution as follows: Dilute a measured quantity of the acid to be standardized to approximately 100 cubic centimeters, heat to boiling and add drop by drop a 10 per cent solution of barium chloride until no further precipitation occurs. Continue the boiling for about five minutes, allow to stand for five hours or longer in a warm place, pour the supernatant liquid on a tared Gooch crucible or an ashless filter, treat the precipitate with 25 to 30 cubic centimeters of boiling water, transfer to the filter and wash with boiling water until the filtrate is free from chloride. Dry, ignite over a Bunsen burner, and weigh as barium sulphate.

(*d*) *Standardization by direct titration.*—Laboratories which interpret protein analyses in terms of the standard alkali, will find that standard alkali solutions can be accurately made up by the use of potassium acid phthalate or benzoic acid as has been shown by Hendrixson.[21]

(*e*) *Standard alkali solution.*—Accurately determine the strength of the standard alkali solution by titration against standard acid, or by the use of potassium acid phthalate or benzoic acid. Standard alkali solutions should not be greater than N/8 in strength.

(*f*) *Sulphuric acid.*—The specific gravity of the acid should be 1.84 and should contain 95 per cent or more sulphuric acid. The acid should be free from nitrates and ammonium salts.

(*g*) *Mercury.*—Red oxide of mercury is preferable, and should be free from nitrates.

[21] See footnote 18.

(*h*) *Granulated zinc or pumice stone.*

(*i*) *Sodium sulphide or sodium thiosulphate.*—Use 50 cubic centimeters of a solution containing 40 grams of sodium sulphide per liter or 50 cubic centimeters of a solution containing 80 grams of sodium thiosulphate per liter.

(*j*) *Powdered potassium or sodium sulphate free from nitrogen:* A mixture of 6 parts of K_2SO_4 and 4 parts of Na_2SO_4 will prevent the formation of the acid cake which forms when Na_2SO^4 is used separately.

(*k*) *Sodium hydroxide solution.*—A saturated solution should be prepared having a specific gravity between 1.43 and 1.48. It is advantageous to dissolve the mercury precipitant in the alkali and add both to the digestion flask at the one operation.

(*l*) *Methyl-red solution.*—Dissolve 1 gram of methyl red in 100 cubic centimeters of 95 per cent alcohol.

APPARATUS

For distillation any suitable flask of about 550 to 800 cubic centimeters capacity may be used. It is fitted with a rubber stopper through which passes the lower end of a Kjeldahl connecting bulb to prevent sodium hydroxide from being carried over mechanically during distillation. The bulb should be about 5 centimeters in diameter and the outlet tube should be of the same diameter as the condenser tube with which the upper end of the bulb is connected. A double-lipped connecting bulb is preferable.

The most essential feature of the digestion and distillation apparatus is the condition of the heating units, for heat is the outstanding factor in the matter of obtaining rapid protein tests.

Providing gas is used as a source of heat, to obtain the most rapid results, the burners on the digestion apparatus should be adjusted so that heat enough is developed to boil away 150 cubic centimeters of water from 200 cubic centimeters in 20 minutes when the 200 cubic centimeters volume is placed in a Kjeldahl flask of 550 cubic centimeters capacity. At this intensity of heat digestion will be complete inside of one hour.

With the usual type of electric heater the period of digestion for those delivering 450 watts should be one hour. The period of digestion should be 45 to 50 minutes with a heater delivering 550 watts, and for those heaters delivering over 600 watts a 40 to 45 minute digestion should be used. Great care should be exercised with heaters of the last-mentioned capacity as acid evaporation at this intensity is very rapid and disastrous results are often obtained with this type of heater.

The gas heaters on the distillation apparatus should be adjusted to develop enough heat to distill over 150 cubic centimeters of water in 25 to 30 minutes.

DETERMINATION

Place 1 gram of the sample in a digestion flask together with approximately 7 to 10 grams of a mixture containing by weight 6 grams of potassium sulphate, 4 grams of sodium sulphate, and one-half gram of red mercuric oxide. Add 20 to 25 cubic centimeters of sulphuric acid, thoroughly mix the acid, salts, and sample together

and digest for one hour. Cool, dilute with 200 cubic centimeters of distilled water, add a few pieces of granulated zinc or pumice stone. Next add sufficient sodium hydroxide and sodium thiosulphate or sodium sulphide solution to make the solution strongly alkaline, and to precipitate the mercury, pouring the alkali down the side of the flask so that it does not immediately mix with the acid solution. Fifty cubic centimeters of solution is usually enough.

Connect the flask with the condenser, mix the contents by shaking and distill until all the ammonia has passed over into a measured quantity of standard acid. The first 150 cubic centimeters of the distillate will generally contain all the ammonia. Titrate with the standard alkali. Calculate, first into terms of nitrogen, later into protein by multiplying the percentage of nitrogen found by 5.7.

Reagents should be tested for absence of nitrogen by making a blank with sugar. The sugar partially reduces any nitrates present that otherwise might escape notice. Deduct any nitrogen found in the reagents from that found under the conditions of the test.

SPECIAL POINTS FOR CONSIDERATION

In making protein tests it appears that efficiency, speed, and inexpensiveness are the most desired essentials. To attain speed an efficient source of heat is necessary. Lacking an efficient source of heat speed can not be obtained, as catalytic agents offer only a limited degree of help in the digestion process. Heat controls the time a sample should be digested.

The period for digestion must necessarily vary according to the heat intensity. With electric heaters, 600 watts capacity, a digestion period of from 40 to 45 minutes is sufficient. Heaters of 550 watts capacity develop sufficient heat to digest the sample completely in 50 to 55 minutes. One hour digestion period is necessary when using heaters of 440 watts capacity. The best heat is developed from gas heaters when they are adjusted so as to evaporate 150 cubic centimeters of water from 200 cubic centimeters of water in 20 minutes. The boiling should take place in a 550-cubic-centimeter Kjeldahl flask.

The present practice of sampling as described should be carefully followed. Having obtained the sample, the matter of keeping its identity can not be overemphasized. If this point is not heeded, it is of little use to make further tests.

As the protein test is usually given along with the numerical grade of sample of wheat, samples for analysis should be cleaned in the manner described.

Practices which lead to the drying out of the grain should not be used, such as taking samples for test after they have been on the trading floor all day subject to drying, handling, and contamination, and the placing of moist wheat in paper sacks preparatory to grinding.

Great care should be taken to retain the moisture in the sample, as changes in moisture induce changes in the protein test results. Differences of 0.6 per cent in protein content have been noted in these investigations caused by changes in moisture content.

Not less than 30 grams of wheat should be ground for protein tests to obtain results representative of the bulk sample. Errors

in protein content of as much as 0.3 per cent will result if smaller samples are used.

From an expense standpoint, provided heat conditions are optimum, the Gunning method can perhaps be used more economically than either of the others, other conditions being equal.

Yellow and red oxides of mercury are equivalent in all quantities as catalytic agents; 0.5 gram gives satisfactory results.

Mercuric sulphate gives slightly lower results than the other two salts studied.

Sodium sulphate and potassium sulphate are equal as catalytic agents. Ten grams of either give best results when 20 cubic centimeters of acid are used. Sodium sulphate, however, has the disadvantage of forming a salt cake with the usual quantities of sulphuric acid used for digestion. This salt cake can be prevented by using a mixture of 60 per cent K_2SO_4 and 40 per cent Na_2SO_4.

Copper sulphate as a catalytic agent is of little value at low intensities of heat.

Wheat should be ground whenever possible. Protein results from samples of whole kernels are irregular when compared to data obtained from carefully ground samples.

There is no difference between electricity and gas as sources of heat, electricity having the advantage in most instances of giving a greater intensity of heat.

Twenty cubic centimeters of concentrated sulphuric acid are necessary to digest and retain all the ammonia liberated from a 1-gram sample of wheat at the intensity of heat usually employed by most laboratories. Twenty-five cubic centimeters are necessary when a 2-gram sample is used.

A 2-gram sample gives slightly higher results than a 1-gram sample. From an error standpoint, the advantage of using a 2-gram sample is not great enough to warrant its use.

Considering the extra time needed to digest a 2-gram sample, the 2-gram sample is undesirable.

The Kjeldahl method is the only method with which wheat samples can be completely oxidized in less than an hour at all intensities of heat. The Gunning method and the Kansas City Protein Referee Board methods are not satisfactory at low heats.

No difference in results can be attributed to methods if the heat is sufficiently intense. Wheat can be completely oxidized in 45 minutes at high heat and in 60 minutes at medium heat, when a 1-gram sample is used. It takes 30 per cent more time to digest a 2-gram sample.

If reductions in the quantities of catalytic agents are made from the figures given in the formulas studied, they will have to be compensated for by more intense or longer heat.

In the distillation process careful attention should be given to the use of traps and to the making of blank determinations.

At least 150 cubic centimeters of distillate should be collected in acid which will hold the equivalent of 35 to 70 milligrams of nitrogen, depending upon whether a 1 or 2 gram sample is used.

There is no choice between sulphuric and hydrochloric as receiving acids.

Boric acid will lose ammonia at 40° C., the loss increasing with the rise in temperature.

Boric acid solutions containing ammonia must be titrated within four hours in order to incur no losses of ammonia.

Sodium thiosulphate is an acceptable substitute for sodium or potassium sulphide as a mercury precipitant.

Careful attention should be given to standardizing normal solutions. Errors in terms of protein equal 0.35 per cent. The error in making such standards is much too large.

The use of potassium acid phthalate as a standard solution is suggested.

Careful attention must be given in the points brought out to assure concordant protein results by different chemists and different protein laboratories.

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